PCT/CA 2004/000837

16 JULY 2004 16.07.04

Office de la propriété intellectuelle du Canada

Un organisme d'Industrie Canada

Canadian Intellectual Property Office

An Agency of Industry Canada

Bureau canadien des brevets

Certification

La présente atteste que les documents ci-joints, dont la liste figure ci-dessous, sont des copies authentiques des documents déposés au Bureau des brevets.

Canadian Patent Office

Certification

This is to certify that the documents attached hereto and identified below are true copies of the documents on file in the Patent Office.

Mémoire descriptif et dessin, de la demande de brevet no: **2,431,425**, tels que déposés le 5 juin 2003, par **ANGIOGÉNE INC.**, cessionnaire de Louis-Georges Guy et Anouk Fortin, ayant pour titre: "ETAS1 Gene Transfer to Improve Cell Therapy".

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

REC'D 11 AUG 2004

WIPO PCT

Agent certificateur/Certifying Officer

16 juillet 2004

Date

Canada

(CIPO 68)
31-03-04

OPIC CIPO

CA 02431425 2003-06-05

Canada Canada
CIPO OPIC
2003/06/05
157- 03
F000001793

EPAS1 GENE TRANSFER TO IMPROVE CELL THERAPY

BACKGROUND OF THE INVENTION

a) Field of the invention

The present invention relates to methods and cells for improving cell implantation and cardiac function. More particularly, the present invention is concerned with the use of nucleotide sequences encoding for EPAS1 for treating coronary and cardiac diseases in mammals.

b) Description of the prior art

Chronic ischemic heart disease is a worldwide health problem of major proportions. According to the American Heart Association, 61 800 000 Americans have at least one type of cardiovascular disease[1]. In particular, coronary heart disease (CHD) cause myocardial infarction (MI) for 7 500 000 American patients and congestive heart failure (CHF) for 4 800 000 American patients. Almost 450 000 deaths in the United States alone were deemed to derive from CHD[1].

Current CHD treatments include medication, percutaneous transluminal coronary angioplasty and coronary artery bypass surgery. These procedures are quite successful to increase blood flow in the myocardium thus reducing ischemia and ameliorating the condition of the patient. However, due to the progressive nature of CHD, the beneficial effects of these procedures are not permanent and new obstructions can occur. Patients that live longer through effective cardiovascular interventions eventually run out of treatment options. Also an important patient population is still refractory to these treatments due to diffuse athereosclerotic diseases and/or small caliber arteries.

Severe and chronic ischemia can cause MI which is an irreversible scarring of the myocardium. This scarring reduces heart contractility and elasticity and consequently

the pumping function, which can then lead to CHF. Treatments available to CHF patients target kidney function and peripheral vasculature to reduce the symptoms but none are treating the scar or increasing pump function of the heart.

An emerging treatment for CHF patients is cellular cardiomyoplasty (CCM), a treatment aiming at reducing the scar and improving heart function. It consists in the injection of cells in the scar, replacing the fibrotic scar by healthy tissue and increasing elasticity. When the injected cells are of muscular origin, they can also contribute to contractility. The net result of this cell therapy is an improvement in heart function. Coupling CCM with therapeutic angiogenesis can improve engraftment of injected cells by increasing the blood supply to the injected cells. Furthermore, the adjacent tissue will benefit from the relief of ischemia. An important limitation of CCM is the high cell death rate at the early stages after implantation. It would be highly desirable to improve cell survival in order to increase efficacy of the treatment.

[illegible] Factors family (HIF). These include HIF-1α (also known as MOP1; see ref. 2 and U.S patents No 5,882,314; 6,020,462 and 6,124,131). Endothelial PAS 1 (EPAS1), (also known as HIF-2α , MOP2, HIF-related factor (HRF) and HLF (HIF-like factor), see ref. 3 and U.S patent No 5,695,963), and the newly discovered HIF-3α (see ref. 4). These factors are highly labile in normal conditions, but are stabilized in response to low oxygen tension. This stabilization allows them to bind to *cis* DNA elements of target genes, and stimulate transcription of hypoxia induced genes that help cell survival in low oxygen conditions. These target genes are implicated in processes such as anaerobic metabolism (glucose transporters and glycolytic enzymes), vasodilatation (inducible nitric oxide synthase (iNOS) and heme oxygenase-1 (HO-1)), increased breathing (tyrosine hydroxylase), erythropoiesis (erythropoietin) and angiogenesis (VEGF).

However, prior to the present invention, it has never been demonstrated or suggested that EPAS1 could induce the expression of cells induced ~~cell survival~~protective genes, nor that EPAS1 modified cell transplanted cells increased -cell survival *in vivo* as indicated by increased metabolic activity. Among the protective genes some improve cell survival, by inhibiting apoptosis or through other mechanisms and others have a cardioprotective activity, preventing scarring of the heart tissue and reducing heart failure. ~~It was shown that adrenomedullin, a cardioprotective gene, was induced by EPAS1(*), but never was it shown for cardiotrophin-1, who~~ ~~which~~ ~~also got some~~ have ~~cardioprotective activity.~~

~~Z: T. Tanaka et al. J Mol Cell Cardiol 2002 Endothelial PAS Domain Protein 1 (EPAS1) induces adrenomedullin gene expression in cardiac myocytes : Role of EPAS1 in an inflammatory response in cardiac myocytes. 34: 739-48.~~

SUMMARY OF THE INVENTION

An object of the present invention is to provide methods and composition of matter for ~~increasing cell survival and/or improving cardiac tissue functions by inducing angiogenesis.~~

~~Still, Aan object of the invention is to provide methods and composition of matter cells~~ for improving cell therapy treatment by increasing cell survival.

Still an object of the invention is to provide methods and composition of matter for improving cardioprotection, which prevents myocardial scarring and reduces heart failure.

HIF 1α and HIF 3α

More particularly, the present invention is concerned with the use of nucleotide sequences encoding EPAS1 transcription factors and functional analogs for treating coronary and cardiac diseases in mammals by CCM. The use of such EPAS1 TFs and its analogs may also be useful in ~~O~~other cell therapy treatment, such as peripheral vascular disease (PVD), neurodegenerative disease including Parkinson's syndrome, muscular dystrophies, stroke, diabetes, hemophilia and others.

An advantage of the present invention is that it provides more effective means for inducing the expression of a plurality of ~~protection~~ protective genes and thereby stimulating cell survival.

The invention is ~~also~~ thus very useful for the treatment of coronary and cardiac diseases in mammals and more particularly for the relief of myocardial ischemia, the regeneration of cardiac tissue subsequent to a myocardial infarction and for the reduction of CHD and also in peripheral vascular disease (PVD).

~~Also, t~~Tissue engineering constructs, such as skin equivalent to treat skin ulcers, would beniefit from an EPAS1 treatement.

Other objects and advantages of the present invention will be apparent upon reading the following non-restrictive description of several preferred embodiments, made with reference to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a bar graph indicating the change in metabolic activity in a scar infarcted area of rat hearts following treatment with autologous myoblasts modified or note with the gene.

DETAILED DESCRIPTION OF THE INVENTION

A) General overview of the invention

An object of the invention is to provide methods and cells for improving cell therapy treatment by increasing cell survival. ~~The methods of the present invention are , and more~~ particularly useful for treating coronary and cardiac diseases in mammals. The invention also provides genetically modified cells expressing a plurality of protective genes.

The invention is based on the use of a nucleotide sequence encoding EPAS1, HIF1α transcription factors2. HIF2α.

As it will be shown in the exemplification section, the present inventors have demonstrated that EPAS1 was stimulating cell survival —genes ~~(also known as angiogenesis-related genes)~~ such as LIF, LIF-R, cardiotrophin 1 and adrenomedullin in myoblasts. ~~It is expected that some or all t~~These genes ~~will also be~~are alsosupposed to be stimulated by EPAS1 in other cell types. The inventors showed, in a rat model of CHF, that EPAS1 modified cells transplanted in the scar tissue survived beter and improved metablic activity.

B) Methods of treatment

According to a first aspect, the invention is directed to a method for inducing in a muscular mammalian cell the expression of at least one ~~angiogenesis-related~~ protective gene, the method comprising the step of introducing and expressing in the cell a nucleic acid sequence encoding a functional EPAS1 transcription factor.

According to another aspect, the invention is directed to a method for ~~increasing the activity of a muscle cell~~improving cell therapy by increasing cell survival and cardioprotection, comprising the step of introducing and expressing in the cell a nucleic acid sequence encoding a functional EPAS1 transcription factor.

~~According to another aspect, the invention is directed to a method for inducing in a cell at least one cardioprotective and/or cell survival gene, the method comprising the step of introducing and expressing in the cell a nucleic acid sequence encoding a functional EPAS1 transcription factor.~~

In a further aspect, the invention is directed to a method for improving cardiac tissue functions of a mammal, comprising the step of providing to the cardiac tissue of the mammal a plurality of genetically modified cells expressing a nucleic acid sequence encoding a functional EPAS1 transcription factor.

~~According to another aspect, the invention is directed to a method for increasing cell survival and implantation following grafting of a tissue such as in a mammalian muscular tissue in a mammalian, comprising the step of providing the grafted cell with a nucleic acid sequence encoding a functional EPAS1 transcription factor.~~

According to the invention, a nucleotide sequence encoding EPAS1 transcription factor is introduced and expressed into a cell. The inventors have found that EPAS1 gene transfer induces the expression of a plurality of protective genes such as LIF, LIF-R, adrenomedullin and cardiotrophin 1.

HIF-1α is described in ref. 2 and in U.S patents No 5,882,314; 6,020,462 and 6,124,131. EPAS1 is described in ref. 3 and U.S patent No 5,692,963. HIF-3α is described in ref. 4 and US provisional application 60/292,630 filed on may 22th 2001. All these documents are incorporated herein by reference.

More preferably, the nucleic acid sequence encoding the transcription ~~factor(s)~~ is a cDNA. The nucleotide sequence may be introduced in the cell or tissue using well known methods. Indeed, the sequence(s) may be introduced directly in the cells of a given tissue, injected in the tissue, or introduced via the transplantation of previously genetically modified compatible cells (see hereinafter). Methods for introducing a nucleotide sequence into eukaryote cells such as mammalian muscular cells or for genetically modifying such cells are well known in the art. For instance, this may be achieved with adenoviral vectors, plasmid DNA transfer (naked DNA or complexed with liposomes) or electroporation. If necessary, a person skilled in the art may look at ref. 11 for a review of myocardial gene therapy methods and to US patent application US20010041679A1 or US patent No. 5,792,453 which provides methods of gene transfer-mediated angiogenesis therapy. Preferably, the level of expression of the transcription factor(s) is such that the protective genes are expressed at a level that is sufficient to ~~induce angiogenesis locally or in surrounding tissue~~improve cell survavil and sustain cardioprotection. For better controlling its expression and selectivity, the transcription factor may be inducible.

In preferred embodiments, a plurality of genetically modified cells are transplanted into the heart of a compatible recipient. Preferably, the transplantation is autologous. More preferably, the transplantation improves the survival of implanted cells. Transplantation methods, are well known in the art. For detailed examples of muscular cell transplantation, one may refer to US patent Nos. 5,602,301 and 6,099,832.

In another preferred embodiment, the muscle cell or the muscular tissue is an ischemic muscular tissue. Accordingly, the expression of at least one protective gene and/or the transplantation of previously genetically modified compatible cells in these ischemic cells or tissue increases tissue function. Also, the efficacy of cell survival and

engraftement being a limiting step, the expression of at least one ~~cell-survival gene and/or cardioprotective~~protective gene is desirable.

In a further aspect, the invention is directed to a genetically modified ~~muscular~~ cell expressing a functional EPAS1 transcription factor. Preferably, the cell is a myoblast, a skeletal muscular cell ~~such as a myoblast~~ or a cardiac cell. The genetically modified ~~C~~cells could also be components of bone marrow, fibroblasts or stem cells. Preferably also, the cell comprises a cDNA encoding the transcription factor. In conditions such as PVD or distrophies, cells should be myoblasts, in stroke and Parkinson's disease, neurons or bone marrow cells and in diabetes, pancreatic islets cells.

As mentioned previously, such cells may be particularly useful when transplanted in a compatible recipient for increasing the metabolic activity of a mammalian muscular tissue, and/or increasing muscular function in CHF or in peripheral vascular disease, locally or in surrounding transplanted tissue.

~~1.~~Of course, the genetically modified cells of the present invention could also be used for the formation of artificial organs or for tissue constructions. Also, other cell types, such as bone marrow cells and their sub-populations, fibroblasts, smooth muscle cells, endothelial cells, endothelial progenitor cells and embryonic stem cells, have other desirable properties for the implantation in other tissue or other type of muscle. Genetic modification of these cells with EPAS1 to improve perfusion and engraftment is also an aspect of the invention.

As it will now be demonstrated by way of an example hereinafter, the present invention is useful for increasing cell survival and tissue function in CHD and in PVD.

EXAMPLES

The following example is illustrative of the wide range of applicability of the present invention and is not intended to limit its scope. Modifications and variations can be made therein without departing from the spirit and scope of the invention. Although any method and material similar or equivalent to those described herein can be used in the practice for testing of the present invention, the preferred methods and materials are described.

EXAMPLE 1: Use of EPAS1 to induce angiogenesis

1) Material and Methods

Adenovirus production

EPAS1/pcDNA3 plasmid was kindly provided by S.L. McKnight[3] and was used to produce adenoviral vectors with the Ad.Easy™ technology using manufacturer methodology (Q-Biogene).

Infection

Early passage human (Clonetics) or rat myoblasts were plated in 100 mm dishes and grown until they reached ~70% confluency. Cells were rinsed with PBS and covered with 4 ml DMEM with 10% fetal calf serum (FCS) and adenoviruses at a MOI of 500. Cells were incubated at 37°C with constant but gentle agitation for 6 hours. 6 ml of DMEM with 10% FCS was added and cells were incubated overnight at 37°C.

Gene chip hybridization

Total RNA was isolated from human myoblasts (Clonetics) infected with either Ad.Null™ (Q-Biogene) or Ad.EPAS1 as described[7]. Probes were prepared and hybridized to Atlas Human 1.2 Array (Clontech) and to 8K Human Atlas Array (Clontech) according to the manufacturer's instructions. The arrays were exposed to phosphorimager screen and analyzed with the Atlas 2.01 software (Clontech).

Cell-survival in infarct heart

Normal or EPAS1 modified rat autologous myoblasts were implanted in infarcted rat hearts 10 days after permanent left anterior descending coronary artery ligation (Myoinfarct™ rats, Charles River Laboratories) by direct myocardial injection of 2 millions cells via a mini-thoracotomy (N=12). Metabolic activity was measured 5 days post ligature and 8 weeks post treatment by injection of ^{18}FDG acquisition using a small animal PET-Scan (Sherbrooke University). FDG uptake in the infarct was quantified and a %.change (post Vs pre treatment) was calculated.

2) Results

Activation of ~~protecteve~~ protective genes by EPAS1 in vitro

To evaluate EPAS1 potential as a cell survival modulator, gene expression was compared in human Myoblast infected either with Ad.EPAS1 or Ad.Null™ using gene chip technology. cDNA probes derived from either cell population was hybridized on a Atlas human 1.2 Array™ or 8K Human Atlas Array (Clontech) assessing expression of almost 1200 genes or 8000 genes. Cell survival and cardioprotective genes were also found to be upregulated by EPAS1: LIF is known to enhance survival of Myoblast, which would be useful in cell therapy. Its receptor, LIF-R, was also stimulated. In the same gene family, cardiotrophin 1 (CT-1) enhances muscle cells survival and protects from heart injury. CT-1 is a survival factor for cardiomyocytes. Adrenomedullin is a potent cardioprotective gene, it has a beneficial effect on left ventricular remodelling after MI and helps prevent heart failure.

Table 1: Genes activated by EPAS1.

Gene	Fold induction	Category
LIF	up	Growth factor
LIF-R	up	Receptor
Adrenomedullin	4.87	Growth factor
CT-1	up	Growth factor

Inductions labeled "up" are representing the activation from a previously undetected gene.

To support the idea that cell survival could be increased by EPAS1, a myoblast implantation in infarct heart study was conducted. It was found that an improved metabolic activity was seen in infarct implanted with EPAS1 modified myoblasts, whereas a deterioration of metabolic activity was seen when unmodified myoblasts were implanted (Figure 1). This result suggest that cell survival was improved, resulting in an increased metabolic activity.

It was shown that adrenomedullin, a cardioprotective gene, was induced by EPAS1[2], but never was it shown for cardiotrophin 1, which also have cardioprotective activityZ: T. Tanaka et al. J Mol Cell Cardiol 2002 Endothelial PAS Domain Protein 1 (EPAS1) induces adrenomedullin gene expression in cardiac myocytes : Role of EPAS1 [illegible]

3) Discussion

The analysis of genes activated by EPAS1 revealed the induction of several protective genes (Table I). These genes play a role in various aspects of survival and cardioprotection and the resulting improved activity is thus expected to be strong and well organized. This is a major advantage compared to the use of a single protective factor.

While several embodiments of the invention have been described, it will be understood that the present invention is capable of further modifications, and this application is intended to cover any variations, uses or adaptations of the invention, following in general the principles of the invention and including such departures from

the present disclosure as to come within knowledge or customary practice in the art to which the invention pertains, and as may be applied to the essential features hereinbefore set forth and falling within the scope of the invention.

REFERENCES

Throughout this paper, reference is made to a number of articles of scientific literature that are listed below and incorporated herein by reference:

1. 2002 Heart and stroke statistical update, American Heart Association.

2. Wang, G.L., Jiang, B.-H., Rue, E.A., and Semenza, G.L. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O_2 tension. *Proc. Natl. Aca. Sci. USA* (1995) **92**: 5510-5514.

3. Tian, H., McKnight, S.L. and Russel, D.W. Endothelial PAS domain protein 1 (EPAS1), a transcription factor selectively expressed in endothelial cells. *Genes & Dev.* (1996) **11**: 72-82.

4. Gu, Y.Z., Moran, S.M., Hogenesch, J.B., Wartman, L. and Bradfield CA. Molecular characterization and chromosomal localization of a third alpha-class hypoxia inducible factor subunit, HIF3alpha. *Gene Expression* (1998) **7**:205-213.

5. Jiang, B.-H., Zheng, J.Z., Leung, S.W., Roe, R. and Semenza, G.L. Transactivation and inhibitory domains of Hypoxia-inducible factor 1α. *J. Biol. Chem.* (1995) **272**: 19253-19260.

6. Vincent, K.A., Shyu, K.-G., Luo, Y., Magner, M., Tio, R.A., Jiang, C., Goldberg, M.A., Akita, G.Y., Gregory, R.J. and Isner, J.M. Angiogenesis is induced in a rabbit model of hindlimb ischemia by naked DNA encoding an HIF-1α/VP16 hybrid transcription factor. *Circulation* (2000) **102**: 2255-2261.

7. Staffa, A., Acheson, N.H. and Cochrane, A. Novel exonic elements that modulate splicing of the human fibronectin EDA exon. *J. Biol. Chem.* (1997) **272**: 33394-401.

8. Tsurumi, Y., Takeshita, S., Chen, D., Kearney, M., Rossow, S.T., Passeri, J., Horowitz, J.R., Symes, J.F. and Isner J.M. Direct intramuscular gene transfer of naked DNA encoding vascular endothelial growth factor augments collateral development and tissue perfusion. *Circulation.* (1996) **94**: 3281-3290.

9. Houle, B., Rochette-Egly, C. and Bradley, W.E. Tumor-suppressive effect of the retinoic acid receptor beta in human epidermoid lung cancer cells. *Proc. Natl. Aca. Sci. USA* (1993) **90**: 985-989.

10. Xia *et al.*, Cancer (2001), **91**:1429-1436.

11. Isner J., Nature (2002), **415**:234-239.

~~While several embodiments of the invention have been described, it will be understood that the present invention is capable of further modifications, and this application is intended to cover any variations, uses or adaptations of the invention, following in general the principles of the invention and including such departures from the present disclosure as to come within knowledge or customary practice in the art to which the invention pertains, and as may be applied to the essential features hereinbefore set forth and falling within the scope of the invention.~~

WHAT IS CLAIMED IS:

1. A method for inducing in a ~~muscular~~ mammalian cell ~~the expression of at least one angiogenesis-related~~ protective gene, the method comprising the step of introducing and expressing in said cell a nucleic acid sequence encoding a functional ~~HIF-2α~~ EPAS1 transcription factor or a functional analog thereof. ~~a functional HIF-3α transcription factor.~~

~~2. The method of claim 1, wherein said transcription factor induces the expression of a plurality of angiogenesis-related genes.~~

2. The method of claim 1, wherein the protective gene is ~~an angiogenesis-related gene.~~a cell survival gene.

3. The method of claim ~~1 or~~ 2, wherein said ~~angiogenesis-related~~cell survival gene is selected from the group consisting of ~~VEGF, IL-8, IL-6, PlGF,~~ LIF, LIF-R, CT-1.

4. The method of claim 1, wherein the protective gene is a cardioprotection gene.

5. The method of claim 2, wherein said cardioprotective gene is CT-1 (cardiotrophin-1).

6.

~~4.~~7. The method of ~~any one of~~ claims 1 ~~to 3~~, wherein said nucleic acid sequence is a cDNA.

8. The method of claim 1 or 2, wherein the cell is a mammalian cell.

9. The method of claim 6, wherein the mammalian cell is selected from the group consisting of myoblast, skeletal muscular cell, cardiomyocyte, smooth muscle cell, bone marrow cell, endothelial cell, endothelial progenitor cell, fibroblast and embryonic stem cell.

~~The method of claim 6, wherein the smooth muscle cell is cardiac cell.~~

~~5.~~10. The method of ~~any one of~~ claims 1 ~~to 4~~, wherein said nucleic acid sequence is introduced into the cell using a method selected from the group consisting of adenoviral infection, and plasmid, cosmid or artificial chromosome transfection or electroporation.

~~6.The method of any one of claims 1 to 5, wherein said cell is a cardiac cell.~~

~~7.The method of any one of claims 17 or to 6 8, wherein the expression of said at least one angiogenesis related gene in a plurality of cardiac cells of said mammal's induce angiogenesis locally or in surrounding tissue, and wherein said angiogenesis improves said mammal's cardiac functions.~~

~~8.The method of any one of claims 1 to 5, wherein said cell is a skeletal muscular cell.~~

~~9.~~11. The method of claim ~~8~~9, further comprising the step of transplanting, into the heart of a compatible recipient, a plurality of said ~~skeletal muscular~~ cells.

~~10.~~12. The method of claim ~~9~~10, wherein said transplantation is autologous.

~~11.~~13. The method of claim 9 ~~or~~ 10 or 11, wherein said transplantation improves the mammal's cardiac functions.

~~12.The method of any one of claims 8~~9 ~~to 11~~2, ~~wherein said cells are transplanted in an amount that is sufficient to induce angiogenesis locally or in surrounding transplanted tissue.~~

~~13.The method of any one of claims 8~~9 ~~to 12, wherein said angiogenesis-related gene is express at a level that is sufficient to induce angiogenesis locally or in surrounding transplanted tissue.~~

14. A method for increasing the metabolic activity of a muscular cell, comprising the step of introducing and expressing in said cell a nucleic acid sequence encoding a functional transcription factor of ~~the Hypoxia Inducible Factor (HIF) family.~~EPAS1.

~~15.The method of claim 14, wherein said functional transcription factor is selected from the group consisting of HIF-1α, HIF-2α, and HIF-3α.~~

15. The method of claim 14 or 15, wherein said transcription factor induces the expression of at least one ~~angiogenesis-related~~cell survival gene selected from the group consisting of VEGF, IL-8, IL-6, PIGF, LIF, LIF-R, CT-1 ~~and adrenomedullin~~ PAI-2 and MMP7.

16. The method of claim 14 or 15, wherein said transcription factor induces the expression of a CT-1, a cardioprotection gene.

17. A method for improving cardiac tissue functions of a mammal, comprising the step of providing to the cardiac tissue of said mammal a plurality of genetically modified cells expressing a nucleic acid sequence encoding a functional ~~HIF-2α transcription factor or a functional HIF-3α~~ EPAS1 transcription factor.

18. The method of claim 17, wherein said genetically modified cells are provided by injecting directly said nucleotide sequence in the cardiac tissue of said mammal.

19. The method of claim 17, wherein said genetically modified cells are provided by transplanting into said cardiac tissue a plurality of ~~skeletal muscular~~ cells genetically modified for expressing said transcription factor, and wherein said ~~skeletal muscular~~ cells originate from a compatible donor.

20. The method of claim 19, wherein said transplantation is autologous.

~~21.~~The method of any one of claims 17 to 20, wherein said transcription factor induce the expression of at least one ~~angiogenesis-related~~cell survival gene selected from the group consisting of ~~VEGF, IL-8, IL-6, PIGF,~~ LIF, LIF-R, CT-1 ~~and adrenomedullin, PAI-2 and MMP7.~~

21. The method of any one of claims 17 to 20, wherein said transcription factor induce the expression of CT-1, a cardioprotection gene.

22.

~~22.The method of any one of claims 17 to 21, for relieving ischemia in coronary heart disease or in peripheral vascular disease.~~

~~23.A method for inducing angiogenesis in a mammalian muscular tissue, comprising the step of providing said tissue with a plurality of genetically modified muscular cells expressing a nucleic acid sequence encoding a functional HIF-2α transcription factor or a functional HIF-3α EPAS1 transcription factor.~~

~~24.The method of claim 23, wherein said genetically modified cells are provided by injecting directly said nucleotide sequence in the cardiac tissue of said mammal.~~

~~25.~~23. The method of claim 23, wherein said genetically modified ~~muscular~~ cells are provided by transplanting into said ~~muscular~~ tissue a plurality of ~~muscular~~ cells genetically modified for expressing said transcription factor, and wherein said ~~muscular~~ cells originate from a compatible donor.

24. The method of claim 25, wherein the tissue is a muscular tissue.

25. The method of claim 26, wherein the muscular tissue is a cardiac tissue.

26. The method of any one of claims 23 to 2~~5~~7, wherein said transcription factor induce in said genetically modified ~~muscular~~ cells the expression of at least one ~~angiogenesis-related~~cell survival gene selected from the group consisting of ~~VEGF, IL-8, IL-6, PIGF,~~ LIF, LIF-R~~,~~ and CT-1~~-and adrenomedullin, PAI-2 and MMP7.~~

27. The method of any one of claims 23 to 27, wherein said transcription factor induce in said genetically modified cells the expression of CT-1, a cardioprotection gene.

~~27.~~28. A genetically modified muscular cell expressing a functional ~~HIF-2α transcription factor or a functional HIF-3α transcription factor~~ EPAS1 transcription factor.

~~28.~~29. The cell of claim 2~~7~~9, wherein said cell is a myoblast, a skeletal muscular cell or a cardiac cell.

~~29.~~30. The cell of claim 2~~7~~9 or ~~28~~ 30, wherein said transcription factor is inducible.

~~30.~~31. The cell of any one of claims 2~~7~~9 to ~~29~~ 31, wherein said transcription factor induce the expression of at least one ~~angiogenesis-related~~cell survival gene selected

from the group consisting ~~of VEGF, IL-8, IL-6, PIGF~~, LIF~~, ~~, LIF-R, CT-1 ~~and adrenomedullin, PAI-2 and MMP7.~~

32. The cell of any one of claims 29 to 31, wherein said transcription factor induce the expression of CT-1, a cardioprotection gene.

33.

34. The cell of any one of claims 29 to 32, wherein it comprises a cDNA encoding said transcription factor.

34. A transformed or transfected cell that contains the nucleic acid of any one of claims 1 to 12.

35. The cell of claim 34, wherein said cell consists of a cell selected from the group consisting of myoblast, mammalian skeletal muscular cells, cardiac cells, bone marrow cells, fibroblasts, smooth muscle cells, endothelial cells, endothelial progenitor cells and embryonic stem cells.

36. A transgenic animal generated from the cell of claim 34 or 35, wherein said nucleic acid is expressed in said transgenic animal.

~~31.The cell of any one of claims 27 to 30, wherein it comprises a cDNA encoding said transcription factor.~~

~~(CLAIMS TAKEN FROM OTHER PATENT TO ADD TO THIS ONE)~~

~~2.A transformed or transfected cell that contains the nucleic acid of any one of claims 1 to 12.~~

~~3.The cell of claim 39, wherein said cell consists of a cell selected from the group consisting of HEK293 cells, Hep3B cells, mammalian skeletal muscular cells, cardiac cells, bone marrow cells, fibroblasts, smooth muscle cells, endothelial cells, endothelial progenitor cells and embryonic stem cells.~~

~~4.A transgenic animal generated from the cell of claim 39 or 40, wherein said nucleic acid is expressed in said transgenic animal.~~

~~5.A method for inducing VEGF expression in a mammalian cell, the method comprising step of introducing and expressing in said cell a nucleic acid sequence encoding polypeptide having the biological activity of a human HIF-2α polypeptide.~~

~~6.The method any one of claims 44 to 47, wherein said cell consists of a cardiac cell located in the heart of a living mammal, and wherein expression of said polypeptide induce angiogenesis in cardiac tissue of said mammal.~~

~~7.The method any one of claims 44 to 47, wherein said cell consists of a muscular cell located in muscular tissue of a living mammal, and wherein expression of said polypeptide induce angiogenesis in the muscular tissue of said mammal.~~

~~8.The method any one of claims 44 to 47, further comprising the step of transplanting said cell in tissue of a compatible mammalian recipient.~~

~~9.The method of claim 50, wherein said cells are transplanted in an amount that is sufficient to induce angiogenesis locally or in surrounding transplanted tissue.~~

~~10.The method of claim 51, wherein said transplanted tissue consists of an ischemic or a non ischemic tissue.~~

~~11.The method of any one of claims 44 to 47, wherein said mammalian cell is a skeletal muscular cell thereby providing a HIF-2α expressing skeletal muscular cell, and wherein said method further comprises the step of transplanting a plurality of said HIF-2α expressing skeletal muscular cells in a cardiac tissue of a compatible mammalian recipient.~~

~~12.The method of any one of claims 50 to 53, wherein said the transplantation step consists of an autologous transplantation and wherein said mammalian recipient is a human.~~

~~13.A method for inducing angiogenesis in a mammalian tissue having a plurality of cells, the method comprising the step of introducing and expressing in at least some of said cells a nucleic acid sequence encoding a polypeptide having the biological activity of a human HIF-2α polypeptide.~~

B

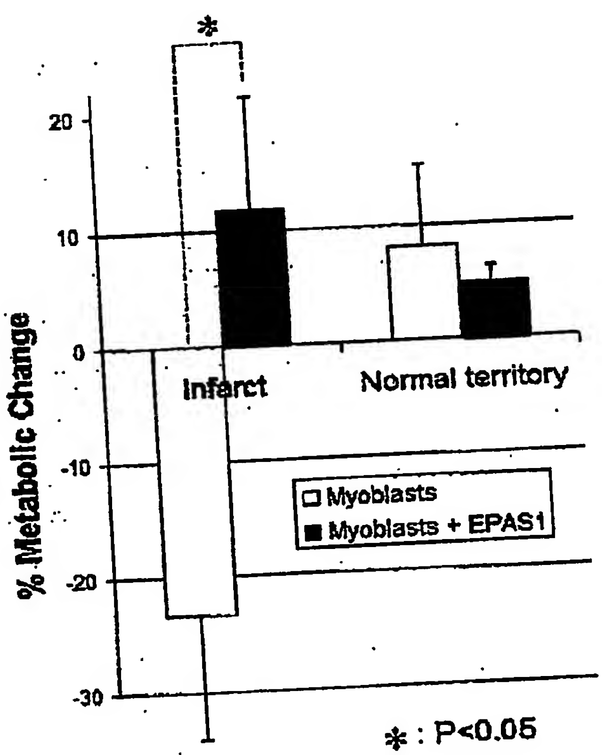
*
20
10
0
-10
-20
-30
% Metabolic Change
Infarct
Normal territory
□ Myoblasts
■ Myoblasts + EPAS1
* : P<0.05

Figure 1